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Flavonoids from the Aerial Parts of *Lonicera japonica*

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Abstract □ Seven flavonoids were isolated from the aerial parts of *Lonicera japonica*. Their structures were characterized as hydnocarpin **1**, quercetin **2**, ochnaflavone **3**, ochnaflavone 4′-*O*-methylether **4**, astragalin **5**, isoquercitrin **6**, and rhoifolin **7** by chemical and spectroscopic evidences.

Keywords □ *Lonicera japonica*, Caprifoliaceae, flavonoid, hydnocarpin, quercetin, ochnaflavone, ochnaflavone 4′-*O*-methylether, astragalin, isoquercitrin, rhoifolin

The aerial parts of *Lonicera japonica* Thunb. (Caprifoliaceae) are used as an antidote, and to treat urinary disorders, fever and headache[1]. Previous authors reported the isolation of various compounds, including flavonoids[2-4], iridoids[5,6], triterpenoid glycosides[7,8] and other compounds[9-11] from this plant. Chemical investigation on this plant parts has led to the isolation of seven flavonoids. This paper deals with their structures.

EXPERIMENTAL METHODS

General experimental procedures

Melting points were taken on a Yanaco micro-melting point apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Perkin-Elmer 841 spectrophotometer and the UV spectra were run with a Varian DMS 200 UV-Vis spectrophotometer. The MS spectra were recorded on a Kratos MS 25 RFA mass spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-300 (300 MHz for ^{1}H-NMR and 75.5 MHz for ^{13}C-NMR) spectrometer with TMS as an internal standard and chemical shifts are given as ppm. Tlc chromatography was performed on pre-coated Kieselgel 60 F_{254} plates (Merck, 5715). Column chromatography was carried out with silica gel (Merck) and Sephadex LH-20 (Pharmacia).

Plant material

The plant material was collected in Kyungbug province in the summer season of 1990. A voucher specimen is deposited in Department of Food and Nutrition, Andong National University.

Extraction and isolation

The dried aerial parts of *L. japonica* (2.4 kg) were extracted with hot MeOH for 6h 3 times to give an extract (573g), which was partitioned with n-hexane (22g), $CHCl_3$ (2.5g), EtOAc (8g) and n-BuOH (20g), successively. The EtOAc fraction was subjected to column chromatography over silica gel using $CHCl_3$-MeOH (gradient), monitored by TLC, to give compounds **1-6** in the order of elution. The n-BuOH fraction was chromatographed with the same manner eluting with $CHCl_3$-MeOH-H_2O (13:7:2, lower layer) to yield compound **7**. Each compound was rechromatographed over Sephadex LH-20 with MeOH as a solvent to give a pure compound.

Hydnocarpin 1

Yellow amorphous powder from MeOH, mp. 259-

Table I. ^{13}C-NMR spectral data for compounds 1-7 in DMSO-d_6

Carbon No.	1	2	Carbon No.	3	4	Carbon No.	5	6	7
C-2	164.2	146.7	C-2	163.0	162.9	C-2	156.4[a]	156.3[a]	164.2
C-3	103.8	135.6	C-3	103.6	104.2	C-3	133.0	133.3	103.1
C-4	181.6	175.7	C-4	181.7	181.7	C-4	177.4	177.4	181.9
C-5	161.3	160.6	C-5	161.4	161.4	C-5	161.2	161.2	161.3[a]
C-6	98.8	98.1	C-6	98.9	98.9	C-6	98.7	98.6	99.3
C-7	162.8	163.8	C-7	164.1	164.3	C-7	164.3	164.1	162.5
C-8	94.0	93.3	C-8	94.0	94.1	C-8	93.7	93.5	94.5
C-9	157.2	156.1	C-9	157.3	157.3	C-9	156.2[a]	156.1[a]	156.9
C-10	103.7	102.9	C-10	103.7	103.8	C-10	104.0	103.9	105.4
C-1′	123.6	121.9	C-1′	122.3	123.7	C-1′	120.9	121.1	120.9
C-2′	114.7	115.0	C-2′	121.2	120.7	C-2′	130.9	115.2	128.5
C-3′	143.5	145.0	C-3′	141.6	142.4	C-3′	115.1	144.8	116.0
C-4′	147.1	147.6	C-4′	153.2	154.5	C-4′	160.0	148.4	161.1[a]
C-5′	117.4	115.5	C-5′	117.9	113.9	C-5′	115.1	116.2	116.0
C-6′	119.8	119.9	C-6′	125.3	125.4	C-6′	130.9	121.5	128.5
C-1″	126.9		C-2″	162.6	162.2	Glc-1	100.9	100.9	97.9
C-2″	111.8		C-3″	104.0	104.1	Glc-2	74.2	74.3	77.0
C-3″	147.6		C-4″	181.7	181.7	Glc-3	77.5	77.5	76.3
C-4″	147.0		C-5″	161.4	161.4	Glc-4	69.9	69.9	70.5
C-5″	115.3		C-6″	98.9	98.9	Glc-5	76.4	76.5	77.2
C-6″	120.5		C-7″	164.2	164.3	Glc-6	60.8	60.9	60.5
C-α	77.9		C-8″	94.0	94.0	Rha-1			100.4
C-β	76.2		C-9″	157.3	157.3	Rha-2			70.3
C-γ	60.0		C-10″	103.8	103.8	Rha-3			69.7
OCH_3	55.7		C-1‴	124.4	124.6	Rha-4			71.8
			C-2‴	128.4	128.4	Rha-5			68.3
			C-3‴	116.1	115.9	Rha-6			18.0
			C-4‴	160.7	160.7				
			C-5‴	116.1	115.9				
			C-6‴	128.4	128.4				
			OCH_3		56.2				

[a]Assignment may be interchangable.

262℃, $FeCl_3$ and Mg/HCl tests: positive. IR ν_{max} (KBr) 3450, 1640, 1280, 1155 cm^{-1}; UV λ_{max} 270, 336 (sh) nm; MS (30 eV) *m/z* (rel. int.) 464 $[M]^+$ (20.3), 446 $[M-H_2O]^+$ (12.3), 435 $[M-CHO]^+$ (1.3), 433 $[M-CH_2OH]^+$ (2.7), 327 (22.3), 286 (100), 258 $[286-CO]^+$ (20.4), 257 (10.8), 180 (31.4), 153 $[A_1+H]^+$ (48.1), 152 $[A_1]^+$ (13.2), 137 $[B_2]^+$ (55.9), 134 $[B_1]^+$ (20.1), 129 (11.3), 124 $[A_1-CO]^+$ (39.4); ^{1}H-NMR (DMSO-d_6) δ: 3.40 (1H, dd, *J*=10, 4.6 Hz, H-γ), 3.60 (1H, dd, *J*=10, 2.3 Hz, H-γ), 3.79 (3H, s, OCH_3), 4.27 (1H, m, H-β), 5.03 (1H, d, *J*=7.8 Hz, H-α), 6.20 (1H, d, *J*=1.9 Hz, H-6), 6.51 (1H, d, *J*=1.9 Hz, H-8), 6.82 (1H, d, *J*=8.1 Hz, H-5″), 6.84 (1H, s, H-3), 6.89 (1H, dd, *J*=8.1, 2.0 Hz, H-6″), 7.04 (1H, d, *J*=2.0 Hz, H-2″), 7.08 (1H, d, *J*=8.6 Hz, H-5′), 7.59 (1H, dd, *J*=8.6, 2.0 Hz, H-6′), 7.65 (1H, d, *J*=2.0 Hz, H-2′), 12.88 (1H, brs, 5-OH); ^{13}C-NMR: see Table I.

Quercetin 2

Yellow amorphous powder from MeOH, mp. > 300℃, $FeCl_3$ and Mg/HCl tests: positive. IR ν_{max} (KBr) 3380 (OH), 1669 (α,β-unsaturated C=O), 1614, 1512 (aromatic C=C) cm^{-1}; UV λ_{max} (MeOH) (log ε) 267 (4.3), 371 (4.5) nm; λ_{max} (MeOH+NaOMe) (log ε) 251 (4.5), 332 (4.5) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 273 (4.5), 337 (3.7), 368 (3.7), 459 (4.6) nm; λ_{max} (MeOH+$AlCl_3$+HCl) (log ε) 269 (4.4), 362

(4.0), 427 (4.5) nm; λ_{max} (MeOH+NaOAc) (log ε) 267 (4.3), 275 (4.4), 325 (4.2), 392 (4.3) nm; λ_{max} (MeOH+NaOAc+H_3BO_3) (log ε) 252 (4.5), 266 (4.3), 388 (4.4) nm; EI-MS *m/z* (rel. int.) 302 $[M]^+$ (100.0), 301 $[M-H]^+$ (16.9), 274 $[M-CO]^+$ (7.0), 273 $[M-HCO]^+$ (8.6), 245 $[273-CO]^+$ (5.4), 153 $[A_1+H]^+$ (10.7), 137 $[B_2]^+$ (19.2), 109 $[137-CO]^+$ (14.4); ^{1}H-NMR (DMSO-d_6) δ: 6.18 (1H, d, *J*=1.9 Hz, H-6), 6.40 (1H, d, *J*=1.9 Hz, H-8), 6.89 (1H, d, *J*=8.5 Hz, H-5′), 7.54 (1H, dd, *J*=2.1, 8.5 Hz, H-6′), 7.67 (1H, d, *J*=2.1 Hz, H-2′), 12.50 (1H, brs, 5-OH); ^{13}C-NMR: see Table I.

***Ochnaflavone* 3**

Pale yellow amorphous powder from MeOH, mp. 233-235℃, $FeCl_3$ and Mg/HCl tests: positive. IR ν_{max} (KBr) 3100-3500 (OH), 1651 (α,β-unsaturated C=O), 1609, 1504 (aromatic C=C), 1357, 1165, 1030, 835 cm^{-1}; UV λ_{max} (MeOH) (log ε) 271 (4.5), 329 (4.5) nm; λ_{max} (MeOH+NaOMe) (log ε) 276 (4.6), 308 (sh, 4.3), 391 (4.5) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 278 (4.4), 299 (4.4), 348 (4.4), 386 (4.4) nm; λ_{max} (MeOH+$AlCl_3$+HCl) (log ε) 280 (4.4), 299 (4.4), 341 (4.4), 386 (4.2) nm; λ_{max} (MeOH+NaOAc) (log ε) 276 (4.5), 308 (4.3), 391 (4.5) nm; λ_{max} (MeOH+NaOAc+H_3BO_3) (log ε) 271 (4.5), 337 (4.4) nm; FAB-MS *m/z* 539 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6) δ: 6.20 (2H, d, *J*=1.8 Hz, H-6, 6″), 6.49 (2H, d, *J*=1.8 Hz, H-8, 8″), 6.86 (2H, s, H-3, 3″), 7.04 (2H, d, *J*=8.8 Hz, H-3‴, 5‴), 7.17 (1H, d, *J*=9.2 Hz, H-5′), 7.90 (1H, dd, *J*=1.8, 9.2 Hz, H-6′), 7.91 (1H, d, *J*=1.8 Hz, H-2′), 8.04 (2H, d, *J*=8.8 Hz, H-2‴, 6‴), 12.86 (1H, s, 5″-OH), 12.89 (1H, s, 5-OH); ^{13}C-NMR: see Table I.

***Ochnaflavone* 4′-*O*-methylether 4**

Pale yellow amorphous powder from MeOH, mp. 297-299℃, $FeCl_3$ and Mg/HCl tests: positive. EI-MS *m/z* (rel. int.) 552 $[M]^+$ (0.8), 430 (1.8), 410 (1.0), 270 (1.2), 256 (1.4), 248 (1.1), 229 (1.1), 213 (1.6), 155 (3.0), 153 (2.3), 152 (1.9), 149 (5.6), 135 (3.4), 129 (4.5), 121 (6.3), 120 (19.5), 107 (8.2), 97 (9.5), 85 (10.1), 83 (12.1), 44 (100.0); ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s, OCH_3), 6.20 (1H, d, *J*=2.1 Hz, H-6″)^a, 6.21 (1H, d, *J*=2.1 Hz, H-6)^a, 6.49 (1H, d, *J*=2.1 Hz, H-8″)^b, 6.51 (1H, d, *J*=2.1 Hz, H-8)^b, 6.86 (1H, s, H-3″)^c, 6.94 (1H, s, H-3)^c, 7.03 (2H, d, *J*=8.9 Hz, H-3‴, 5‴), 7.40 (1H, d, *J*=8.1 Hz, H-5′), 7.95 (1H, d, *J*=2.3 Hz, H-2′), 8.04 (2H, d, *J*=8.9 Hz, H-2‴, 6‴), 8.06 (1H, dd, *J*=2.3, 8.1 Hz, H-6′), 12.85 (2H, s, 5, 5″-OH). ^{a,b,c}Assignments may be interchangable; ^{13}C-NMR: see Table I.

***Astragalin* 5**

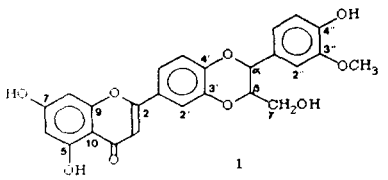
Yellow needles from MeOH, mp. 183-185℃, $FeCl_3$, Mg/HCl and Molisch tests: positive. IR ν_{max} (KBr) 3435 (OH), 1660 (α,β-unsaturated C=O), 1605, 1575, 1505 (aromatic C=C), 1100-1000 (glycosidic C-O) cm $^{-1}$; UV λ_{max} (MeOH) (log ε) 267 (4.3), 309 (4.1), 350 (4.2) nm; λ_{max} (MeOH+NaOMe) (log ε) 275 (4.4), 326 (4.1), 401 (4.4) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 274 (4.3), 305 (4.0), 352 (4.2), 397 (4.2) nm; λ_{max} (MeOH+$AlCl_3$+HCl) (log ε) 275 (4.3), 302 (4.0), 346 (4.2), 396 (4.1) nm; λ_{max} (MeOH+NaOAc) (log ε) 275 (4.4), 309 (4.1), 384 (4.2) nm; λ_{max} (MeOH+NaOAc+H_3BO_3) (log ε) 268 (4.3), 305 (4.1) 353 (4.2) nm; ^{1}H-NMR (DMSO-d_6) δ: 5.37 (1H, d, *J*=7.2 Hz, Glc H-1), 6.16 (1H, d, *J*=1.7 Hz, H-6), 6.39 (1H, d, *J*=1.7 Hz, H-8), 6.86 (2H, d, *J*=8.8 Hz, H-3′, 5′), 7.95 (2H, d, *J*=8.8 Hz, H-2′, 6′), 12.60 (1H, brs, 5-OH); ^{13}C-NMR: see Table I.

***Isoquercitrin* 6**

Yellow needles from MeOH, mp. 236℃, $FeCl_3$, Mg/HCl and Molisch tests: positive. IR ν_{max} (KBr) 3385 (OH), 1658 (α,β-unsaturated C=O), 1260, 1176, 1075, 1035, 810 cm^{-1}; UV λ_{max} (MeOH) (log ε) 257 (4.4), 268 (sh, 4.4), 300 (sh, 4.1), 362 (4.3) nm; λ_{max} (MeOH+NaOMe) (log ε) 273 (4.5), 335 (sh, 4.1), 415 (4.5) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 275 (4.5), 305 (sh, 4.0), 337 (sh, 3.9), 440 (4.5) nm; λ_{max} (MeOH+$AlCl_3$+HCl) (log ε) 270 (4.4), 300 (sh, 4.0), 365 (sh, 4.2), 404 (4.3) nm; λ_{max} (MeOH+NaOAc) (log ε) 275 (4.5), 325 (4.1), 400 (4.3) nm; λ_{max} (MeOH+NaOAc+H_3BO_3) (log ε) 265 (4.5), 387 (4.4) nm; ^{1}H-NMR (DMSO-d_6) δ: 5.43 (1H, d, *J*=7.4 Hz, Glc H-1), 6.18 (1H, d, *J*=1.8 Hz, H-6), 6.38 (1H, d, *J*=1.8 Hz, H-8), 6.84 (1H, d, *J*=8.2 Hz, H-5′), 7.56 (1H, dd, *J*=2.1, 8.2 Hz, H-6′), 7.59 (1H, d, *J*=2.1 Hz, H-2′), 12.60 (1H, s, 5-OH); ^{13}C-NMR: see Table I.

***Rhoifolin* 7**

Yellow needles from MeOH, mp. 260-263℃, $FeCl_3$, Mg/HCl and Molisch tests: positive. IR ν_{max} (KBr) 3340 (OH), 1660 (α,β-unsaturated C=O), 1075 cm^{-1}; UV λ_{max} (MeOH) (log ε) 268 (4.4), 333 (4.5) nm; λ_{max} (MeOH+NaOMe) (log ε) 245 (sh, 4.2), 267 (4.2),



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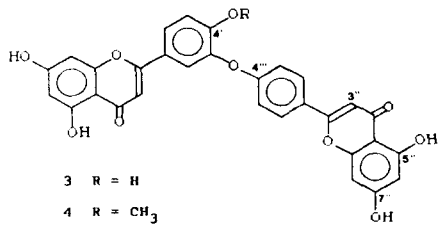
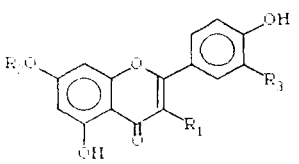


3 R = H

4 R = CH_3



2 R_1 = R_3 = OH R_2 = H

5 R_1 = OGlc R_2 = R_3 = H

6 R_1 = OGlc R_2 = H R_3 = OH

7 R_1 = H R_2 = Rha$^{1\rightarrow2}$Glc R_3 = H

300 (sh, 3.7), 386 (4.7) nm; λ_{max} (MeOH+$AlCl_3$) (log ε) 275 (4.3), 300 (4.2), 348 (4.5), 383 (4.4) nm; λ_{max} (MeOH-$AlCl_3$+HCl) (log ε) 276 (4.3), 299 (4.2), 342 (4.5), 380 (4.3) nm; λ_{max} (MeOH+NaOAc) (log ε) 257 (sh, 4.2), 267 (4.3), 354 (4.2), 387 (4.2) nm; λ_{max} (MeOH+NaOAc+H_3BO_3) (log ε) 267 (4.3), 342 (4.5) nm; ^{1}H-NNR (DMSO-d_6) δ: 1.20 (3H, d, J=6.2 Hz, Rha CH_3), 5.09 (1H, s, Rha H-1), 5.22 (1H, d, J=7.0 Hz, Glc H-1), 6.38 (1H, d, J=1.8 Hz, H-6), 6.79 (1H, d, J=1.8 Hz, H-8), 6.84 (1H, s, H-3), 6.95 (2H, d, J=8.7 Hz, H-3″, 5″), 7.93 (2H, d, J=8.7 Hz, H-2″, 6″), 12.95 (1H, s, 5-OH); ^{13}C-NMR: see Table I.

Acid hydrolysis of 5, 6 and 7

Compounds **5**, **6** and **7** (30 mg each) in 60% dioxane-H_2SO_4 (5%, 5 m*l*) were separately refluxed for 2h, and each reaction mixture was poured onto iced water and filtered. Each precipitate was purified by recrystallization from MeOH to afford kaempferol from **5**, quercetin from **6** and apigenin from **7**, which were identified by direct comparison with authentic samples. Each filtrate was neutralized with $BaCO_3$, filtered and concentrated in vacuo. D-glucose from **5** and **6**, and D-glucose and L-rhamnose from **7** were detected by TLC ($CHCl_3$-MeOH-H_2O =26:14:5).

Permethylation of 3 and 4

Compound **3** (30 mg) was permethylated with NaH (50 mg) and CH_3I (0.5 m*l*) by the Brimacombe's method[12]. The reaction product was purified by column chromatography with $CHCl_3$ to afford a penta-*O*-methylether of compound **3** as amorphous solid. mp. 166-168℃; EI-MS (30 eV) *m/z* (rel. int.) 608 $[M]^+$ (8.2), 593 $[M-CH_3]^+$ (11.8), 430 (3.5), 281 (1.5), 280 (1.7), 267 (1.5), 202 (1.7), 195 (1.6), 181 (2.2), 167 (3.6), 149 (7.7), 120 (21.5), 44 (100); ^{1}H-NMR ($CDCl_3$) δ: 3.88 (3H, s, CH_3), 3.89 (3H, s, CH_3), 3.90 (3H, s, CH_3), 3.94 (3H, s, CH_3), 3.95 (3H, s, CH_3), 6.37 (1H, d, J=2.3 Hz, H-6)^a, 6.38 (1H, d, J=2.3 Hz, H-6″)^a, 6.52 (1H, d, J=2.3 Hz, H-8)^b, 6.55 (1H, d, J=2.3 Hz, H-8″)^b, 6.57 (1H, s, H-3)^c, 6.60 (1H, s, H-3″)^c, 7.03 (2H, d, J=8.9 Hz, H-3‴, 5‴), 7.13 (1H, d, J=8.7 Hz, H-5″), 7.62 (1H, d, J=2.2 Hz, H-2′), 7.74 (1H, dd, J=2.2, 8.7 Hz, H-6′), 7.83 (2H, d, J=8.9 Hz, H-2‴, 6‴). ^{a,b,c}Assignments may be interchangable. Compound **4** (20 mg) was also permethylated in the same manner as above. Both reaction products were identical in all respects.

RESULTS AND DISCUSSION

Column chromatography of EtOAc and BuOH fractions of MeOH extract afforded seven compound, three of which were identified as quercetin **2**, astragalin **4** and isoquercitrin **5** by comparison of spectral data with those of the reported in literature[13] as well as direct comparison with authentic samples.

Compound **1** exhibited UV spectrum similar to those of flavones[14] and showed absorption bands due to an α,β-unsaturated C=O (1640 cm^{-1}) and hydroxyl groups (3450 cm^{-1}) in its IR spectrum. The ^{1}H-NNR spectral data of **1** indicated signals due to a luteolin derivative at δ 6.20 (1H, d, J=1.9 Hz), 6.51 (1H, d, J=1.9 Hz), 6.84 (1H, s), 7.65 (1H, d, J=2.0 Hz), 7.59 (1H, dd, J=2.0, 8.6 Hz), 7.08 (1H, d, J=8.6 Hz) and 12.88 (1H, brs) ppm. In addition, other signals at δ 6.82 (1H, d, J=8.1 Hz), 6.89 (1H, dd, J=2.0, 8.1 Hz), 7.04 (1H, d, J=2.0 Hz), 3.79 (3H, s), 4.27 (1H, m), 5.03 (1H, d, J=7.8 Hz), 3.40 (1H,

dd, J=4.6, 10 Hz), 3.60 (1H, dd, J=2.3, 10 Hz) were reminiscent of coniferyl alcohol moiety[17] in compound **1**. The mode of linkage of this two units can be visualised as involving condensation of coniferyl alcohol with luteolin giving rise to the 1,4-dioxane system, which was also apparent from the observation of the mass spectrum. The mass spectrum of **1** showed a molecular ion at *m/z* 464, and characteristic fragments originated in the flavonoid part of the molecule due to cleavage of the 1,4-dioxane ring gave the ions at *m/z* 286 [luteolin moiety]$^+$, 258 [286-CO]$^+$, 153 [A_1+H]$^+$, 152 [A_1]$^+$, 137 [B_2]$^+$, 134 [B_1]$^+$ and 124 [A_1-CO]$^+$ indicating the presence of a luteolin moiety. Other fragments at *m/z* 180, 137, 124 and 91 were characteristic of the coniferyl alcohol moiety[15]. Therefore, it is obvious that compound **1** seems to be a flavonolignan derivative carrying a 1,4-benzodioxane nucleus such as silybin and silandrin[16] and americanin A[17]. Based on the above results, the structure of **1** was determined to be hydnocarpin which was first isolated from *Hydnocarpus wightiana*[18]. Later, its structure was revised as **1**[19]. The structure of **1** was finally verified by comparison of the ^{13}C-NMR data with those of the reported in literature[20]. This is the first report of the isolation of this compound from Caprifoliaceous plants.

Compound **3** showed broad hydroxyl groups and α,β-unsaturated C=O absorptions at 3100-3500 and 1651 cm^{-1}, respectively, in its IR spectrum. The UV maxima at 271 and 329 nm were very similar to those of reported apigenin derivatives. It exhibited a bathochromic shift with $AlCl_3$, $AlCl_3$+HCl and NaOMe in band I and with NaOAc in band II which indicated the presence of free hydroxyl groups at C-5, 7 and 4′. It showed a protonated molecular ion at *m/z* 539 [M+H]$^+$ in the FAB-MS spectrum, indicating that **3** consists of two apigenin moieties. The ^{1}H-NMR spectrum of **3** showed two *meta*-coupled protons at δ 6.20 (2H) and 6.49 (2H) ppm together with 1,3,4-trisubstituted [δ 7.91 (1H, d, J=1.8 Hz); 7.17 (1H, d, J=9.2 Hz); 7.90 (1H, dd, J=1.8, 9.2 Hz)] and 1,4-disubstituted benzene ring protons [δ 7.04 (2H, d, J=8.8 Hz); 8.04 (2H, d, J=8.8 Hz)]. These observations were further supported by the ^{1}H-^{1}H COSY spectrum of permethylether derivative, mp. 166-168°, which was prepared by Brimacombe's method[12]. Therefore compound **3** should be comprised of two apigenin moieties linked at the C-3′ and 4‴-positions. In the light of above observations, the structure of compound **3** was elucidated as 5,7,4′,5″,7″-pentahydroxy-3′,4‴-biflavonyl ether (ochnaflavone)[20].

Compound **4** showed close similar spectral data to those of **3**. It showed a molecular ion at *m/z* 552 in the EI-MS spectrum and a methoxyl singlet signal at δ 3.85 in its ^{1}H-NMR spectrum, suggesting that this compound is a monomethyl derivative of compound **3**. Permethylation of **4**, prepared by Brimacombe's method, gave a penta-*O*-methylether derivative identical in all respects with the penta-*O*-methylether prepared from ochnaflavone **3**. The *O*-methylation induced shifts at C-4′ in compound **4** were observed at the C-5′ and C-1′ carbon atoms[22]. The substantial upfield (−4.0 ppm) and the relatively weak (+1.4) downfield shifts observed on the C-5′ and on the C-1′, respectively, of **4** compared to ochnaflavone **3** clearly indicated that the C-4′ hydroxyl group was methylated. On the basis of the above results, the structure of **4** was characterized as ochnaflavone 4′-*O*-methylether[21].

Compound **7** showed positive results for flavonoid glycoside and gave glucose and rhamnose on acid hydrolysis together with apigenin. Comparison of UV spectra of **7** with apigenin indicated that the position of glycosidation is at C-7 hydroxyl group[14]. The interglycosidic linkage was determined by ^{13}C-NMR spectroscopy. It showed a set of ^{13}C-NMR signals for terminal rhamnose moiety and glycosidation shift for C-2 chemical shift of inner glucose. Thus the interglycosidic linkage in rhamnoglucose moiety is 1→2, neohesperidose. From the above results, the structure of **7** was assigned as apigenin 7-neohesperidoside (rhoifolin) which was isolated from *Rhus succedanea*[23].

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